

PTO/SB/21 (02-04)

Approved for use through 07/31/2006. OMB 0651-0031

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

**TRANSMITTAL  
FORM**

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission

7

Application Number	09/547,501
Filing Date	APRIL 12, 2000
First Named Inventor	CHRISTIAN
Art Unit	1617
Examiner Name	SHAOJIA A. JIANG
Attorney Docket Number	IMI-002

**ENCLOSURES (Check all that apply)**

- |  |   |   |
|--|---|---|
| <input type="checkbox"/> Fee Transmittal Form                                | <input type="checkbox"/> Drawing(s)                                       | <input type="checkbox"/> After Allowance communication to Technology Center (TC)        |
| <input type="checkbox"/> Fee Attached  | <input type="checkbox"/> Licensing-related Papers                         | <input type="checkbox"/> Appeal Communication to Board of Appeals and Interferences     |
| <input checked="" type="checkbox"/> Amendment/Reply                          | <input type="checkbox"/> Petition   | <input type="checkbox"/> Appeal Communication to TC (Appeal Notice, Brief, Reply Brief) |
| <input type="checkbox"/> After Final   | <input type="checkbox"/> Petition to Convert to a Provisional Application | <input type="checkbox"/> Proprietary Information  |
| <input type="checkbox"/> Affidavits/declaration(s)                           | <input type="checkbox"/> Power of Attorney, Revocation                    | <input type="checkbox"/> Status Letter  |
| <input type="checkbox"/> Extension of Time Request                           | <input type="checkbox"/> Change of Correspondence Address                 | <input type="checkbox"/> Other Enclosure(s) (please identify below):                    |
| <input type="checkbox"/> Express Abandonment Request                         | <input type="checkbox"/> Terminal Disclaimer                              |   |
| <input type="checkbox"/> Information Disclosure Statement                    | <input type="checkbox"/> Request for Refund                               |   |
| <input type="checkbox"/> Certified Copy of Priority Document(s)              | <input type="checkbox"/> CD, Number of CD(s) _____                        |   |
| <input type="checkbox"/> Response to Missing Parts/Incomplete Application    |   |   |
| <input type="checkbox"/> Response to Missing Parts under 37 CFR 1.52 or 1.53 |   |   |

**Remarks**

Supplemental Response in re. August 26, 2004 RCE; Courtesy copy Likhoshevstov et al. ; No Fee is req

**SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT**

Firm or Individual name	BioMedPatent John S. Sundsmo, Ph.D., 34,446
-------------------------	--

Signature	
-----------	--

Date	September 2, 2004
------	-------------------

**CERTIFICATE OF TRANSMISSION/MAILING**

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below.

Typed or printed name	JOHN S. SUNDSMO, PH.D., 34,446
-----------------------	--------------------------------

Signature		Date	September 2, 2004
-----------	--	------	-------------------

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

AF/1617  
JFW

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Christian

Serial No: 09/547,501

Filed: April 12, 2000

Title: NOVEL PHARMACEUTICAL AGENTS CONTAINING CARBOHYDRATE MOIETIES AND METHODS OF THEIR PREPARATION AND USE



Agent Docket No. IMI-002

Group Art Unit: 1617

Examiner: Shaojia A. Jiang

SUPPLEMENTAL RESPONSE TO OFFICE ACTION  
AND COURTESY COPY OF LIKHOSHERSTOV ET AL.

Vista, California 92085  
September 2, 2004

TO THE COMMISSIONER OF PATENTS AND TRADEMARKS:

**Office Action:** Courtesy copy of Likhosherstov et al. and Supplemental response are now made in regard to the Office Action ("Action") carrying a mailing date of February 26, 2004 setting a 3 month period for response expiring May 26, 2004, i.e., extended with payment of fee under 37 C.F.R. § 1.136(a) to expire on August 26, 2004; and Request for Continued Examination filed with Response and Amendment on August 26, 2004.

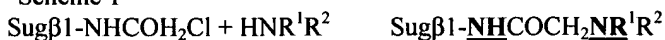
**Documents Transmitted:** Copy of Likhosherstov et al. recently acquired from CNRS ( Cedex, Paris, France); and, supplemental response.

**Supplemental Response In Re. Likhosherstov et al.**

With apologies to the Examiner, Applicant encountered some considerable difficulties in locating a copy of the Likhosherstov et al. article cited by the office as a Chem. Abstract. With the full text now received from France, some additional and clarifying response seems appropriate. A courtesy copy is transmitted herewith in the event that the Examiner may have encountered similar difficulties.

In regard to motivation in Likhosherstov et al., at page 1244 appears the following: namely,

"Scheme 1



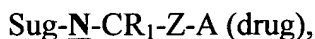
1a-e

Sug = D-Gal(β1-4)D-Gluc (a); D-GlcNAc (b); D-Gal (c); D-Man (d); D-Glc (e)" (page 1244, right column, lines 1-5; emphasis added);

"Alkylation of secondary amines was studied using morpholine and piperazine and its derivatives (N-methylpiperazine and 1,4-diazabicyclo[4.3.0]nonane ). Piperazine is a well-known anthelmintic, and its structural fragment is a part of anesthetic, psychotropic, and antitumor drugs. **Lectins specific to the residues of β-D-glucosamine** are widespread on the surface of animal cells. Therefore, for the synthesis of glycoconjugates we used derivatives of these monosaccharides and the disaccharide lactose." (page 1244, right column, lines 8-17; emphasis added);

"The introduction of mono- and oligosaccharide residues into the molecules of various physiologically active compounds and drugs presents considerable interest due to the possibility of controlled change of their interactions with receptors and of target-directed transport to particular cells, which contain specific carbohydrate-binding proteins (**lectins**) on their surface." (page 1244, left column, lines 1-7; emphasis added).

Likhosherstov et al. teaches away from the claimed invention, i.e., the instant reaction product of claimed invention as compared with the disclosure above, is as follows: namely,



wherein R<sub>1</sub> and Z (when present) comprise lower alkyl or substituted lower alkyl.

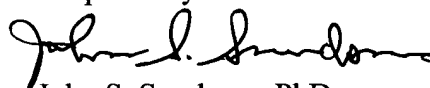
Likhosherstov et al. motivates binding at N-acetyl-glucosaminyl- "lectins"/"receptors". Chemical properties required for binding at those receptors, i.e., the presence of N-acetyl-glucosyl-NH<sub>2</sub>, are thus motivated by Likhosherstov et al. and not the requisite properties for blood brain barrier transport of glycosyl compounds. The difference makes a difference, in that (as related in prior responses and in the instant Specification) sugar transporters are stereo- and anomer-specific and glucose, not glucosamine or NAc-glucosamine, is required for binding at glucose blood brain barrier transporters (GLUT). As related previously, even galactose (differing in anomeric position of

a hydroxyl) is not transported. Motivating different chemistry, thus, makes a considerable and significant difference in the expected outcome. Thus, different chemistry, different motivation, and expectation of different success are all features of Likhosherstov et al. Obviousness must be certain. Applicant believes Likhosherstov et al. teaches away.

### **Concluding Remarks**

In light of the amendments to the claims and remarks transmitted August 26, 2004, as well as the supplemental remarks herein, removal of the rejections under 35 U.S.C. § 103 is respectfully requested. If any issues remain which can be expeditiously addressed in teleconference, the Examiner is urged to contact Applicant's agent at 760-806-3385 (office) or 615-423-3850 (mobile).

Respectfully submitted:



John S. Sundsmo, PhD  
Registration No.: 34,446

# Glycoconjugates of amines: alkylation of primary and secondary amines with *N*-chloroacetyl- $\beta$ -glycopyranosylamines

L. M. Likhoshesterov,\* O. S. Novikova, and V. N. Shibaev

N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences,  
47 Leninsky prosp., 117913 Moscow, Russian Federation.  
Fax: +7 (095) 135 5328. E-mail: shiba@ioc.ac.ru



Efficient monoalkylation of a series of primary and secondary amines was demonstrated with the use of *N*-chloroacetylglucosylamines derived from D-glucose, D-galactose, D-mannose, *N*-acetyl-D-glucosamine, and lactose. The reaction was shown to be useful for incorporation of carbohydrate residues into physiologically active compounds. Glycoconjugates of some derivatives of piperazine, 2-phenylethylamine, tryptamine, and important biogenic amines (norephedrine, octopamine, dopamine) were prepared.

**Key words:** glycoconjugates, *N*-chloroacetylglucosylamines, piperazine, 2-phenylethylamine, tryptamine, norephedrine, octopamine, dopamine.

The introduction of mono- and oligosaccharide residues into the molecules of various physiologically active compounds and drugs presents considerable interest due to the possibility of controlled change of their interaction with receptors and of target-directed transport to particular cells, which contain specific carbohydrate-binding proteins (lectins) on their surface.

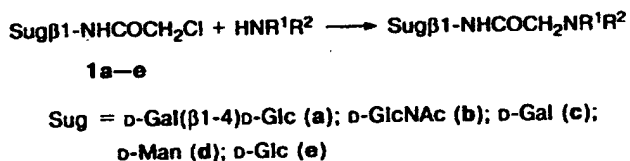
Modification of the initial compounds with glycosylamine derivatives appears to be a promising approach to this type of construction of drug precursors ("prodrugs"). The glycosylamines can be easily prepared from either monosaccharides<sup>1-4</sup> or complex oligosaccharides including the products of cleavage of natural *N*-glycoproteins.<sup>5-7</sup> The use of glycosylamines for the synthesis of glycoconjugates generally involves their *N*-acylation and subsequent modification based on reactions of functional groups present in the acyl residue (see reviews<sup>8,9</sup>).

A convenient variant of this approach is transformation of glycosylamines into *N*-haloacetylglucosylamines,<sup>7,10,11</sup> which are subsequently used to modify peptides and proteins at the SH groups.<sup>10,11</sup> The transformation of *N*-chloroacetylglucosylamines of oligosaccharides into *N*-glycylglucosylamines after the reaction with (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub>, and the use of the products for preparing various glycoconjugates containing fluorescent labels, biotin residues, palmitic acid, and also bovine serum albumin have been described.<sup>7,12,13</sup> However, the possibilities of using *N*-haloacetylglucosylamines for introduction of carbohydrate residues into other types of biologically active compounds still remain little studied.

In this work, we studied the reaction of some *N*-chloroacetyl- $\beta$ -glycopyranosylamines **1a–e**, described in our previous publication,<sup>4</sup> with a number of primary and secondary amines (Scheme 1).

This reaction was used to alkylate a number of physiologically active compounds, which made it pos-

Scheme 1



sible to prepare previously unknown glycoconjugates **2–8** (Scheme 2).

Alkylation of secondary amines was studied using morpholine and piperazine and its derivatives (*N*-methylpiperazine and 1,4-diazabicyclo[4.3.0]nonane). Piperazine is a well-known anthelmintic, and its structural fragment is a part of anesthetic, psychotropic, and antitumor drugs. Lectins specific to the residues of  $\beta$ -D-galactose and *N*-acetyl-D-glucosamine are widespread on the surface of animal cells.<sup>14</sup> Therefore, for the synthesis of glycoconjugates we used derivatives of these monosaccharides and the disaccharide lactose.

We found that secondary amines are smoothly alkylated upon treatment with chloroacetyl derivatives of glycosylamines (molar ratio 2 : 1) at 70 °C in MeOH or aqueous MeOH. The course of the reaction can be conveniently monitored by paper electrophoresis. After the reaction has been carried out for 3 h, the conversion of the alkylating reagent was 85–90%. Under the conditions chosen, we did not observe noticeable cleavage of the *N*-glycosylamide bond, whose lability in an alkaline medium has been noted previously for a glucose derivative.<sup>15</sup> The reaction products were separated from the initial *N*-chloroacetylglucosylamines by the cation exchange chromatography and additionally purified by crystallization or chromatography on Al<sub>2</sub>O<sub>3</sub>; the yields of conjugates **2**, **3c**, and **4** were about 65%.

Translated from *Izvestiya Akademii Nauk. Seriya Khimicheskaya*, No. 6, pp. 1244–1247, June, 1998.

1066-5285/98/4706-1214 \$20.00 © 1998 Plenum Publishing Corporation

and dissolved in 2 mL of H<sub>2</sub>O. The solution was applied to a column (1×13 cm) packed with the Amberlite IRC-50 cation exchanger (H<sup>+</sup>). The column was washed with 100 mL of H<sub>2</sub>O and 100 mL of 0.5 M aqueous pyridine. The fractions that contained product 2, according to the electrophoresis data, were combined and concentrated to dryness, and the residue was recrystallized from MeOH. Yield 0.29 g (63%), m.p. 214–216 °C,  $[\alpha]_D^{20} +4.8^\circ$  (c 1, H<sub>2</sub>O). Found (%): C, 45.11; H, 7.11; N, 6.10; H<sub>2</sub>O, 2.80. C<sub>18</sub>H<sub>32</sub>N<sub>2</sub>O<sub>12</sub> · 1/2 H<sub>2</sub>O. Calculated (%): C, 45.27; H, 6.96; N, 5.87; H<sub>2</sub>O, 1.89. <sup>1</sup>H NMR, δ: 2.62 (br.s, 4 H, CH<sub>2</sub>NCH<sub>2</sub>); 3.22 (br.s, 2 H, COCH<sub>2</sub>); 3.42–3.97 (m, 16 H); 4.47 (d, 1 H, H(1) Gal, *J* = 8 Hz); 5.03 (d, 1 H, H(1) Glc, *J* = 9 Hz). <sup>13</sup>C NMR, δ: 54.5 (CH<sub>2</sub>NCH<sub>2</sub>); 61.7, 62.3, 62.9 (2 CH<sub>2</sub>OH, COCH<sub>2</sub>); 68.1 (CH<sub>2</sub>OCH<sub>2</sub>); 70.4, 72.8, 73.3, 74.4, 76.95, 77.2, 78.3, 79.6 (C(2)–C(5) Glc and Gal), 80.8 (C(1) Glc); 104.7 (C(1) Gal); 175.0 (CO).

***N*-Piperazinoacetyl-2-acetamido-2-deoxy-β-D-glucopyranosylamine (3a) and *N*-piperazinoacetyl-β-D-galactopyranosylamine (3b).** Piperazine (0.87 g, 10 mmol) was added to a solution of *N*-chloroacetyl-2-acetamido-2-deoxy-β-D-glucopyranosylamine (1b) (0.3 g, 1 mmol) in 7 mL of MeOH or *N*-chloroacetyl-β-D-galactopyranosylamine (1c) (0.25 g, 1 mmol) in 12 mL of 70% aqueous MeOH. The mixture was heated for 3 h at 70 °C. The MeOH was evaporated, and the residue was dissolved in 20 mL of H<sub>2</sub>O; 35 mL of the Dowex 50Wx8 cation exchanger (H<sup>+</sup>) was added, and the mixture was stirred for 1 h. The resin was filtered off and washed with 400 mL of H<sub>2</sub>O and 300 mL of 1.5 M NH<sub>4</sub>OH. The alkaline fractions were concentrated to dryness, and the reaction products were isolated from the residue.

Compound 3a was isolated by chromatography on a column (2.8×11 cm) packed with Al<sub>2</sub>O<sub>3</sub> (propan-2-ol → MeOH) followed by crystallization (MeOH–propan-2-ol). Yield 0.2 g (58%), m.p. 218–219 °C,  $[\alpha]_D^{20} +26.7^\circ$  (c 1, H<sub>2</sub>O). Found (%): C, 48.42; H, 7.55; N, 16.25. C<sub>14</sub>H<sub>26</sub>N<sub>4</sub>O<sub>6</sub>. Calculated (%): C, 48.54; H, 7.57; N, 16.17. <sup>1</sup>H NMR, δ: 2.05 (s, 3 H, CH<sub>3</sub>CO); 2.58 (br.s, 4 H, CH<sub>2</sub>NCH<sub>2</sub>); 2.92 (br.s, 4 H, CH<sub>2</sub>NHCH<sub>2</sub>); 3.20 (s, 2 H, COCH<sub>2</sub>); 3.51–3.98 (m, 6 H); 5.15 (d, 1 H, H(1), *J* = 9 Hz).

Compound 3b was isolated by crystallization (MeOH–propan-2-ol). Yield 0.21 g (58%), m.p. 219–221 °C (decomp.),  $[\alpha]_D^{20} +14.5^\circ$  (c 1, H<sub>2</sub>O). Found (%): C, 47.24; H, 7.63; N, 13.52. C<sub>12</sub>H<sub>23</sub>N<sub>3</sub>O<sub>6</sub>. Calculated (%): C, 47.20; H, 7.59; N, 13.76. <sup>1</sup>H NMR, δ: 2.60 (br.s, 4 H, CH<sub>2</sub>NCH<sub>2</sub>); 2.95 (br.s, 4 H, CH<sub>2</sub>NHCH<sub>2</sub>); 3.25 (br.s, 2 H, COCH<sub>2</sub>); 3.66–3.87 (m, 5 H); 4.04 (m, 1 H, H(4)); 5.03 (d, 1 H, H(1), *J* = 9 Hz).

***N*-(4-Methylpiperazin-1-ylacetyl)-2-acetamido-2-deoxy-β-D-glucopyranosylamine (3c).** A mixture of *N*-chloroacetyl-2-acetamido-2-deoxy-β-D-glucopyranosylamine (1b) (0.45 g, 1.5 mmol) and *N*-methylpiperazine (0.3 mL, 3 mmol) in 5 mL of MeOH was heated for 3 h at 70 °C. The solvent was evaporated, and the residue was dissolved in 15 mL of H<sub>2</sub>O; 12 mL of the Dowex 50Wx8 cation exchanger (H<sup>+</sup>) was added, and the mixture was stirred for 1.5 h. The resin was filtered off, washed with 200 mL of H<sub>2</sub>O and then with 200 mL of 1.5 M NH<sub>4</sub>OH. The alkaline fractions were evaporated to dryness. Recrystallization of the residue (MeOH–acetone) gave compound 3c. Yield 0.4 g (74%), m.p. 205–207 °C,  $[\alpha]_D^{20} +24.7^\circ$  (c 1, H<sub>2</sub>O). Found (%): C, 47.64; H, 8.08; N, 15.37; H<sub>2</sub>O, 4.56. C<sub>15</sub>H<sub>28</sub>N<sub>4</sub>O<sub>6</sub> · H<sub>2</sub>O. Calculated (%): C, 47.61; H, 7.99; N, 14.81; H<sub>2</sub>O, 4.76. <sup>1</sup>H NMR, δ: 2.06 (s, 3 H, CH<sub>3</sub>CO); 2.30 (s, 3 H, NCH<sub>3</sub>); 2.57 (br.s, 8 H, 4 CH<sub>2</sub>); 3.19 (br.s, 2 H, COCH<sub>2</sub>); 3.50–3.96 (m, 6 H); 5.13 (d, 1 H, H(1), *J* = 9.5 Hz).

***N*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-2-(1,4-diazabicyclo[4.3.0]non-4-yl)acetamide (4).** Compound 4 was prepared similarly to 3c from 1,4-diazabicyclo[4.3.0]nonane (0.375 g, 3 mmol)<sup>18</sup> and purified by chromatography on a column (2.5×8 cm) packed with Al<sub>2</sub>O<sub>3</sub> (propan-2-ol → MeOH) followed by crystallization (MeOH–ether). Yield 0.5 g (65%), m.p. 150–151 °C,  $[\alpha]_D^{20} +21.7^\circ$  (c 1, H<sub>2</sub>O). Found (%): C, 50.40; H, 7.87; N, 14.52; H<sub>2</sub>O, 4.56. C<sub>17</sub>H<sub>30</sub>N<sub>4</sub>O<sub>6</sub> · H<sub>2</sub>O. Calculated (%): C, 50.48; H, 7.97; N, 13.85; H<sub>2</sub>O, 4.45. <sup>1</sup>H NMR, δ: 1.38 (m, 1 H); 1.71–1.97 (m, 3 H); 2.02 (s, 3 H, CH<sub>3</sub>CO); 2.05–2.47 (m, 5 H); 2.71–3.06 (m, 4 H); 3.20 (s, 2 H, COCH<sub>2</sub>); 3.47–3.59 (m, 2 H, H(4,5) GlcN); 3.65 (t, 1 H, H(3) GlcN); 3.77 (dd, 1 H, H(6a) GlcN); 3.84–3.94 (m, 2 H, H(2b,6b) GlcN); 5.10 (d, 1 H, H(1) GlcN, *J* = 9.5 Hz). <sup>13</sup>C NMR, δ: 21.5 (CH<sub>2</sub>); 23.1 (CH<sub>3</sub>); 27.8 (CH<sub>2</sub>); 51.2 (CH<sub>2</sub>); 52.5 (CH<sub>2</sub>); 53.3 (CH<sub>2</sub>); 55.3 (C(2) GlcN); 57.6 (CH<sub>2</sub>); 61.3 (CH<sub>2</sub>); 61.6 (CH<sub>2</sub>); 63.1 (NCH); 70.7 (C(4) GlcN); 75.3 (C(3) GlcN); 78.8 (C(5) GlcN); 79.4 (C(1) GlcN); 174.6 (CO); 175.7 (CO).

***N*-(*N*-Phenethylglycyl)-2-acetamido-2-deoxy-β-D-glucopyranosylamine (5a) and *N*-(*N*-phenethylglycyl)-β-D-mannopyranosylamine (5b).** 2-Phenylethylamine (0.4 mL, 3 mmol) and 10 mL MeOH were added to a solution of *N*-chloroacetyl-2-acetamido-2-deoxy-β-D-glucopyranosylamine (1b) (0.3 g, 1 mmol) or *N*-chloroacetyl-β-D-mannopyranosylamine (1d) (0.25 g, 1 mmol) in 2 mL of DMSO, and the mixture was heated for 10 h at 70 °C. The MeOH was evaporated, and the residue was diluted with 25 mL of toluene. The resulting oily product was washed 3 times with toluene and ether, and dissolved in 10 mL of H<sub>2</sub>O. The solution was stirred for 1.5 h with 10 mL of the Dowex 50Wx8 cation exchanger (H<sup>+</sup>). The resin was filtered off, washed with 150 mL of H<sub>2</sub>O, 150 mL of 1.5 M NH<sub>4</sub>OH, and 150 mL of 1.5 M NH<sub>4</sub>OH containing 6% Py. The alkaline fractions were concentrated to dryness, and the reaction products were isolated from the residue.

Compound 5a was isolated by crystallization (MeOH–propan-2-ol). Yield 0.28 g (71%), m.p. 223–225 °C,  $[\alpha]_D^{20} +21.4^\circ$  (c 1, H<sub>2</sub>O). Found (%): C, 55.41; H, 7.11; N, 11.02; H<sub>2</sub>O, 2.12. C<sub>18</sub>H<sub>27</sub>N<sub>3</sub>O<sub>6</sub> · 1/2 H<sub>2</sub>O. Calculated (%): C, 55.37; H, 7.23; N, 10.76; H<sub>2</sub>O, 2.31. <sup>1</sup>H NMR, δ: 1.95 (s, 3 H, CH<sub>3</sub>CO); 2.84 (m, 4 H, CH<sub>2</sub>CH<sub>2</sub>); 3.34 (br.s, 2 H, COCH<sub>2</sub>); 3.49–3.60 (m, 2 H, GlcN); 3.66 (t, 1 H, H(3) GlcN); 3.75–3.95 (m, 3 H, GlcN); 5.12 (d, 1 H, H(1) GlcN, *J* = 9.5 Hz); 7.32–7.50 (m, 5 H, Ar).

Compound 5b was isolated by chromatography on a column (1.4×12 cm) with Al<sub>2</sub>O<sub>3</sub> (acetone → propan-2-ol → MeOH). The yield of the amorphous compound was 0.22 g (65%),  $[\alpha]_D^{20} -24.8^\circ$  (c 1, H<sub>2</sub>O). Found (%): C, 56.71; H, 7.20; N, 8.23. C<sub>16</sub>H<sub>24</sub>N<sub>2</sub>O<sub>6</sub>. Calculated (%): C, 56.46; H, 7.11; N, 8.23. <sup>1</sup>H NMR, δ: 2.90 (m, 4 H, CH<sub>2</sub>CH<sub>2</sub>); 3.46 (br.s, 2 H, COCH<sub>2</sub>); 3.48 (m, 1 H, H(5) Man); 3.62 (t, 1 H, H(4) Man); 3.68–3.78 (m, 2 H, Man); 3.88–3.98 (m, 2 H, Man); 5.24 (br.s, 1 H, H(1) Man); 7.30–7.48 (m, 5 H, Ar).

***N*-(*N*-[2-(Indol-3-yl)ethyl]glycyl)-β-D-galactopyranosylamine (6)** was synthesized similarly to compound 5 from *N*-chloroacetyl-β-D-galactopyranosylamine (1c) (0.25 g, 1 mmol) and tryptamine (0.48 g, 3 mmol) in a mixture of 2 mL of DMSO and 18 mL of MeOH over a period of 30 h; prior to the treatment with the cation exchanger, the aqueous solution was decolorized by carbon. Product 6 was crystallized from H<sub>2</sub>O. Yield 0.2 g (54%), m.p. 142–143 °C,  $[\alpha]_D^{20} +15.4^\circ$  (c 1, CH<sub>3</sub>OH). Found (%): C, 54.90; H, 7.17; N, 10.58; H<sub>2</sub>O, 3.55. C<sub>18</sub>H<sub>25</sub>N<sub>3</sub>O<sub>6</sub> · H<sub>2</sub>O. Calculated (%): C, 54.40; H, 6.85; N, 10.57; H<sub>2</sub>O, 4.53. <sup>1</sup>H NMR (CD<sub>3</sub>OD), δ: 2.95 (br.s, 4 H, CH<sub>2</sub>CH<sub>2</sub>); 3.54–3.68 (m, 3 H, Gal);

3.71–3.78 (m, 2 H, Gal); 3.94 (m, 1 H, H(4) Gal); 4.92 (d, 1 H, H(1) Gal,  $J = 9$  Hz); 6.99–7.15 (m, 2 H, Ar); 7.12 (s, 1 H, Ar); 7.37 (d, 1 H, Ar,  $J = 8$  Hz); 7.58 (d, 1 H, Ar,  $J = 8$  Hz).  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ ),  $\delta$ : 26.7 ( $\text{CH}_2\text{Ar}$ ); 51.2 ( $\text{NCH}_2$ ); 53.0 ( $\text{NCH}_2$ ); 62.8 (C(6) Gal); 70.7 (C(4) Gal); 71.8 (C(2) Gal); 76.0 (C(3) Gal); 78.5 (C(5) Gal); 81.6 (C(1) Gal); 112.5, 113.8, 119.6, 119.8, 122.6, 123.8, 129.0, 138.5 (8 C, Ar); 175.4 (CO).

*N*-{*N*-[(1*S*,2*R*)-1-Hydroxy-1-phenylprop-2-yl]glycyl}-4-*O*-( $\beta$ -D-galactopyranosyl)- $\beta$ -D-glucopyranosylamine (7a) and *N*-{*N*-[DL-2-hydroxy-2-(4-hydroxyphenyl)ethyl]glycyl}-4-*O*-( $\beta$ -D-galactopyranosyl)- $\beta$ -D-glucopyranosylamine (7b) was synthesized similarly to 5 from *N*-chloroacetyl-4-*O*-( $\beta$ -D-galactopyranosyl)- $\beta$ -D-glucopyranosylamine monohydrate (1a) (0.22 g, 0.5 mmol) and D-norephedrine hydrochloride (0.28 g, 1.5 mmol) or DL-octopamine hydrochloride (0.28 g, 1.5 mmol) in the presence of  $\text{Et}_3\text{N}$  (0.11 mL, 1.5 mmol) in a mixture of 1 mL of DMSO and 5 mL of MeOH. The reaction duration was 22 h. The residue obtained after concentration of the alkaline fractions resulting from ion exchange chromatography was treated with acetone (5  $\times$  15 mL) in order to remove the initial amine (in the case of DL-octopamine, hot acetone was used) and dissolved in 5 mL of  $\text{H}_2\text{O}$ . The solution was applied to a column (4  $\times$  100 cm) with Sephadex G-25 (fine), and the column was washed with water (1 L) and then with 0.1 *M* AcOH. The fractions containing the products were combined, concentrated to dryness, dissolved in  $\text{H}_2\text{O}$ , and lyophilized, and the amorphous residue was dried *in vacuo* over KOH.

Compound 7a was obtained in a yield of 0.16 g (61%),  $[\alpha]_{\text{D}}^{20} +5.2^\circ$  (c 1,  $\text{H}_2\text{O}$ ). Found (%): C, 51.42; H, 6.67; N, 5.23.  $\text{C}_{23}\text{H}_{36}\text{N}_2\text{O}_{12}$ . Calculated (%): C, 51.87; H, 6.81; N, 5.26.  $^1\text{H}$  NMR,  $\delta$ : 1.18 (d, 3 H,  $\text{CH}_3$ ); 3.10 (m, 1 H, NCH); 3.48 (br.s, 2 H,  $\text{COCH}_2$ ); 3.40–4.05 (m, 12 H, Glc, Gal); 4.55 (d, 1 H, H(1) Gal,  $J = 8$  Hz); 4.70 (d, 1 H, CHAr,  $J = 6$  Hz); 5.03 (d, 1 H, H(1) Glc,  $J = 9$  Hz); 7.46–7.58 (m, 5 H, Ar).

Compound 7b was obtained as the corresponding acetate by precipitation with ether from a solution in MeOH. Yield 0.21 g (79%)  $[\alpha]_{\text{D}}^{20} +4.5^\circ$  (c 1,  $\text{H}_2\text{O}$ ). Found (%): C, 48.10; H, 6.34; N, 4.51.  $\text{C}_{21}\text{H}_{34}\text{N}_2\text{O}_{11} \cdot \text{CH}_3\text{COOH}$ . Calculated (%): C, 48.48; H, 6.44; N, 4.71.  $^1\text{H}$  NMR,  $\delta$ : 1.90 (s, 3 H,  $\text{CH}_3\text{CO}$ ); 2.88 (m, 2 H, NCH<sub>2</sub>); 3.38 (br.s, 2 H,  $\text{COCH}_2$ ); 3.47–3.94 (m, 13 H); 4.44 (d, 1 H, H(1) Gal,  $J = 8$  Hz); 4.98 (d, 1 H, H(1) Glc,  $J = 9$  Hz); 6.89 (d, 2 H, Ar,  $J = 8$  Hz); 7.32 (d, 2 H, Ar,  $J = 8$  Hz).

*N*-{*N*-[2-(3,4-Dihydroxyphenyl)ethyl]glycyl}- $\beta$ -D-glucopyranosylamine hydrochloride (8). Triethylamine (0.147 mL, 2 mmol) was added to a solution of *N*-chloroacetyl- $\beta$ -D-glucopyranosylamine (1e) (0.13 g, 0.5 mmol) and dopamine (0.38 g, 2 mmol) in a mixture of 1.3 mL of DMSO and 2.6 mL of MeOH. The mixture was heated for 2.5 h at 70  $^\circ\text{C}$  and poured in 45 mL of toluene, and the precipitate was washed with toluene (3  $\times$  15 mL) and ether and dissolved in 3 mL of  $\text{H}_2\text{O}$ . To the solution, 4 mL of 0.5 *M* HCl was added, and the solution was applied to a column (5  $\times$  90 cm) with Sephadex G-15. The column was washed with  $\text{H}_2\text{O}$  (1.5 L) and then with 0.1 *M* AcOH. The fractions containing the reaction products were combined, concentrated *in vacuo*, and lyophilized, and the amorphous residue was dried over  $\text{P}_2\text{O}_5$ . The yield of product 8 was 0.115 g (56%);  $[\alpha]_{\text{D}}^{20} -9.6^\circ$  (c 1,  $\text{H}_2\text{O}$ ).

Found (%): C, 46.60; H, 6.64; N, 6.23, Cl, 9.07.  $\text{C}_{16}\text{H}_{24}\text{N}_2\text{O}_8 \cdot \text{HCl}$ . Calculated (%): C, 47.00; H, 6.16; N, 6.85, Cl, 8.67.  $^1\text{H}$  NMR,  $\delta$ : 2.95 (t, 2 H,  $\text{CH}_2\text{Ar}$ ,  $J = 7$  Hz); 3.36 (t, 2 H, NCH<sub>2</sub>,  $J = 7$  Hz); 3.39–3.59 (m, 4 H, Glc); 3.73 (dd, 1 H, H(6a) Glc); 3.89 (dd, 1 H, H(6b) Glc); 3.98 (br.s, 2 H,  $\text{COCH}_2$ ); 5.03 (d, 1 H, H(1),  $J = 9$ ); 6.77 (d, 1 H, Ar,  $J = 8$ ); 6.86 (s, 1 H, Ar); 6.42 (d, 1 H, Ar,  $J = 8$ ).

This work was supported by the Russian Foundation for Basic Research (Project No. 97-03-32989).

## References

1. L. M. Likhoshesterov, O. S. Novikova, V. A. Derevitskaya, and N. K. Kochetkov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1986, 1663 [*Bull. Acad. Sci. USSR, Div. Chem. Sci.*, 1986, 35, 1512 (Engl. Transl.)].
2. E. Kallin, H. Lonn, T. Norberg, and M. Elofsson, *J. Carbohydr. Chem.*, 1989, 8, 597.
3. D. Vetter and M. A. Gallop, *Bioconjugate Chem.*, 1995, 6, 316.
4. L. M. Likhoshesterov, O. S. Novikova, V. N. Shibaev, and N. K. Kochetkov, *Izv. Akad. Nauk, Ser. Khim.*, 1996, 1848 [*Russ. Chem. Bull.*, 1996, 45, 1760 (Engl. Transl.)].
5. V. E. Piskarev, L. M. Likhoshesterov, N. F. Sepetov, V. A. Derevitskaya, and N. K. Kochetkov, *Bioorg. Khim. [Bioorg. Chem.]*, 1988, 14, 1704 (in Russian).
6. V. E. Piskarev, L. M. Likhoshesterov, and V. A. Derevitskaya, in *Lectins — Biology, Biochemistry, Clinical Biochemistry*, Eds. J. Kocourek and D. J. L. Freed, Sigma Chemical Company, St. Louis, 1990, 7, 197.
7. I. D. Manger, T. W. Rademacher, and R. A. Dwek, *Biochemistry*, 1992, 31, 10724.
8. E. Kallin, in *Neoglycoconjugates: Preparation and Applications*, Eds. Y. C. Lee and R. T. Lee, Acad. Press, San Diego, 1994, 199.
9. S. Y. C. Wong, *Curr. Opin. Struct. Biol.*, 1995, 5, 599.
10. T. S. Black, L. Kiss, D. Tull, and S. G. Withers, *Carbohydr. Res.*, 1993, 250, 195.
11. S. Y. C. Wong, G. R. Guile, R. A. Dwek, and G. Arsequell, *Biochem. J.*, 1994, 300, 843.
12. I. D. Manger, S. Y. C. Wong, T. W. Rademacher, and R. A. Dwek, *Biochemistry*, 1992, 31, 10733.
13. S. Y. C. Wong, I. D. Manger, G. R. Guile, T. W. Rademacher, and R. A. Dwek, *Biochem. J.*, 1993, 296, 817.
14. H. J. Gabius, *Eur. J. Biochem.*, 1997, 243, 543.
15. T. Takeda, Y. Sugiura, Y. Ogihara, and S. Shibata, *Can. J. Chem.*, 1980, 58, 2600.
16. V. R. Hegde, P. Dai, C. Ladislav, M. G. Patel, M. S. Puar, and J. A. Pachter, *Bioorg. Med. Chem. Lett.*, 1997, 7, 1207.
17. R. Polt, F. Porreca, L. Z. Szabo, E. J. Bilsky, P. Davis, T. J. Abbruscato, T. P. Davis, R. Horvath, H. I. Yamamura, and V. J. Hruby, *Proc. Natl. Acad. Sci. USA*, 1994, 91, 7114.
18. L. S. Nazarova, A. M. Likhoshesterov, K. S. Raevskii, and A. P. Skoldinov, *Khim.-farm. zhurn.*, 1976, 10, 88 [*Chem. Pharm. J.*, 1976, 10 (Engl. Transl.)].

Received December 5, 1997